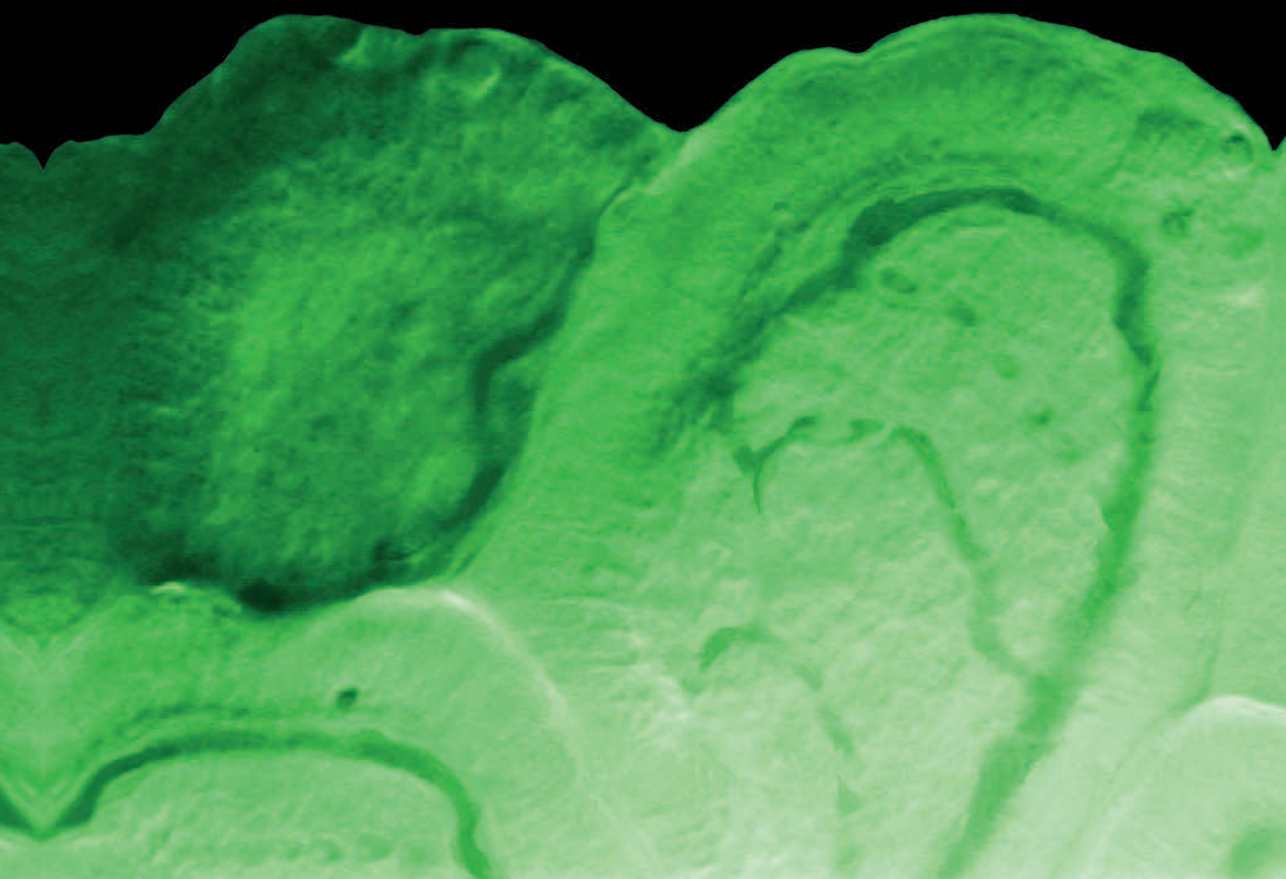
Arginine and the microcirculation

Chapter 13

Valorization



In the following paragraphs we will describe the value is of our work for society and the economy. Furthermore, we will highlight which target population will benefit from our newly developed model and therapeutic strategy. Finally we will shortly describe the innovation of our model and the utilization of our knowledge.

Social and economic value of the current thesis

Sepsis is a major health problem, which are associated with high rates of morbidity and mortality. The reported incidence varies between 75 to 300 per 100,000 patients worldwide [1], with mortality rates of approximately 25%. However, if complicated by multiple organ failure due to an impaired microcirculation, the mortality rates doubles [2]. This results in high socio-economic costs. As described in **chapter 2** of this thesis, an imbalance in NO production by NOS3 and NOS2 is hypothesized to play a key role in the development of multiple organ failure during sepsis and inflammatory conditions [3,4]. Therefore, restoring NO production from arginine by the proper cells and enzymes in these conditions has been the subject of numerous studies over the past decades [5-14]. Thus far, there is no definite treatment, so that the search for good therapeutic interventions is still ongoing. Previous nutritional interventions focussed on arginine supplementation, but the outcomes of these treatments differed widely [14-25], with either increased [21], unchanged [9,14,19], or decreased morbidity and mortality.

The disparate outcomes of the human studies appear to result from the experimental animal models used to simulate and treat sepsis. Thus far, the acute sepsis model, which takes only a few hours, is usually used, but this model does not cause arginine deficiency. Therefore, the first goal of the current thesis was the development of an experimental model that mimics the human arginine-deficient state during endotoxemia. As described in detail in **Chapter 4**, we developed a mouse model with an 18h infusion of endotoxcin that causes a decreased arginine availability and a diminished NO production and decreased microcirculation in jejunal tissue as found in septic human patients. Since our model mimics the human situation better with respect to the functional arginine deficiency than previously used models, new interventions tested in this model may

be better suitable to have outcomes that better predict efficacy in humans that the previously used acute models.

To develop a new therapeutic approach for sepsis, we first investigated whether arginine would be a suitable substrate in our model. As hypothesized, the results of our study show that arginine supplementation did not restore arginine availability and microcirculation during endotoxemia. Therefore, we conclude that arginine is not a suitable substrate for the treatment of these disturbances in NO production.

In this thesis we further showed that L-citrulline supplementation is a good therapeutic strategy to restore the arginine availability in the cells of the jejunum during experimental endotoxemia. L-citrulline supplementation even resulted in an improved microcirculation and maintenance of the gastro-intestinal barrier in healthy athletes. Based on these beneficial results of L-citrulline supplementation, the findings during our study are potentially of high social and economic value for the medical society and the world of sports.

Target population

The results of this thesis are of interest to several target groups within the research field and health care. At first, the results presented in this thesis provide new insights for the development of specific nutritional supplements for patients with a disturbed arginine metabolism, such as septic patients. Therefore, the data of thesis may be of special interest to pharmaceutical companies to develop new nutritional formulas for clinical usage in patients with disturbances in arginine metabolism. In, line these results are important for health care workers in the field of intensive care medicine, which deal with these septic patients daily. However, this arginine deficiency is not only present in inflammatory conditions, but is also present in abnormal fracture healing [26], disturbed wound healing [27]and cardiovascular diseases [28-30]. Therefore, the results of this thesis, will be of interest to a much broader audience, including surgeons, cardiologists, and investigators in the biomedical field with special interest in endothelial dysfunction and microcirculation. Also, athletes and investigators in the field of sport medicine are likely to be interested, based on the beneficial results of L-citrulline supplementation during strenuous exercise.

Knowledge utilization

The knowledge obtained from our research in the area of nutritional supplementation in patients with arginine deficiency will be valorized through its application in future studies focused on improved metabolism during inflammatory conditions. The knowledge received from this thesis can already be incorporated in the current standard treatment of critical ill patients, focusing on enhancement of the microcirculation. Furthermore, as already mentioned in the previous paragraph the insights from our studies will help to develop nutritional strategies targeting the endothelial function is several different pathophysiological states. This eventually will lead to a less social economic cost based on a decreased hospitalization and diminished morbidity and mortality.

Innovation

The search for a good therapeutic strategy to maintain a functional microcirculation during sepsis and endotoxemia has been the subject of many different studies over the past decades. However, experimental studies used acute sepsis models, which are not a reliable model to study the human situation. In this regard, the present thesis exhibits various novel findings and insights. First, the experimental model described in this thesis is the first model, which uses a prolonged endotoxemia to test the therapeutic strategies targeting the arginine availability and metabolism. Furthermore, our results are the first to show that citrulline is a suitable substrate to improve arginine availability and microcirculatory function during inflammatory conditions, and, therefore, may be a promising new therapeutic strategy in patients. The novelty of the model and therapeutic approach has not gone unnoticed, as we were awarded the International Sepsis Forum award of the European Society of Intensive Care Medicine (ESICM) in 2010 for the best new research on therapeutic interventions in the treatment of human sepsis. These studies will serve as basis for future studies that address arginine and NO deficiencies in human patients, especially in the (critically) ill.

References

1. Simmons J, Pittet JF (2015) The coagulopathy of acute sepsis. Curr Opin Anaesthesiol.
2. Mayr FB, Yende S, Angus DC (2014) Epidemiology of severe sepsis. Virulence 5: 4-11.
3. Trzeciak S, Cinel I, Phillip Dellinger R, Shapiro NI, Arnold RC, et al. (2008) Resuscitating the microcirculation in sepsis: the central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. Acad Emerg Med 15: 399-413.
4. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, et al. (2007) Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med 49: 88-98, 98 e81-82.
5. Bruins MJ (2001) L-Arginine treatment during acute and longterm endotoxemia in the pig : effects on jejunal motility and interorgan protein, arginine and nitric oxide metabolism 1-243.
6. Bruins MJ, Soeters PB, Lamers WH, Deutz NE (2002) L-arginine supplementation in pigs decreases liver protein turnover and increases hindquarter protein turnover both during and after endotoxemia. Am J Clin Nutr 75: 1031-1044.
7. Bruins MJ, Soeters PB, Lamers WH, Meijer AJ, Deutz NE (2002) L-arginine supplementation in hyperdynamic endotoxemic pigs: effect on nitric oxide synthesis by the different organs. Crit Care Med 30: 508-517.
8. Poeze M, Bruins MJ, Kessels F, Luiking YC, Lamers WH, et al. (2011) Effects of L-arginine pretreatment on nitric oxide metabolism and hepatosplanchnic perfusion during porcine endotoxemia. Am J Clin Nutr 93: 1237-1247.
9. Luiking YC, Poeze M, Deutz NE (2015) Arginine infusion in patients with septic shock increases nitric oxide production without haemodynamic instability. Clin Sci 128: 57-67.
10. Luiking YC, Poeze M, Ramsay G, Deutz NE (2005) The role of arginine in infection and sepsis. JPEN J Parenter Enteral Nutr 29: S70-74.
11. Barbul A, Lazarou SA, Efron DT, Wasserkrug HL, Efron G (1990) Arginine enhances wound healing and lymphocyte immune responses in humans. Surgery 108: 331-336; discussion 336-337.
12. Schaffer MR, Tantry U, Gross SS, Wasserburg HL, Barbul A (1996) Nitric oxide regulates wound healing. J Surg Res 63: 237-240.
13. Vissers YL, Debats IB, Luiking YC, Jalan R, van der Hulst RR, et al. (2004) Pros and cons of L-arginine supplementation in disease. Nutr Res Rev 17: 193-210.

14. Preiser JC, Berre PJ, Van Gossum A, Cynober L, Vray B, et al. (2001) Metabolic effects of arginine addition to the enteral feeding of critically ill patients. JPEN J Parenter Enteral Nutr 25: 182-187.
15. Bode-Boger SM, Boger RH, Galland A, Tsikas D, Frolich JC (1998) L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. Br J Clin Pharmacol 46: 489-497.
16. Bertolini G, Luciani D, Biolo G (2007) Immunonutrition in septic patients: a philosophical view of the current situation. Clin Nutr 26: 25-29.
17. Deutz NE (2008) The 2007 ESPEN Sir David Cuthbertson Lecture: amino acids between and within organs. The glutamate-glutamine-citrulline-arginine pathway. Clin Nutr 27: 321-327.
18. Grover Z, Tubman R, McGuire W (2007) Glutamine supplementation for young infants with severe gastrointestinal disease. Cochrane Database Syst Rev: CD005947.
19. Kieft H, Roos AN, van Drunen JD, Bindels AJ, Bindels JG, et al. (2005) Clinical outcome of immunonutrition in a heterogeneous intensive care population. Intensive Care Med 31: 524-532.
20. Tsuei BJ, Bernard AC, Barksdale AR, Rockich AK, Meier CF, et al. (2005) Supplemental enteral arginine is metabolized to ornithine in injured patients. J Surg Res 123: 17-24.
21. Bertolini G, Iapichino G, Radrizzani D, Facchini R, Simini B, et al. (2003) Early enteral immunonutrition in patients with severe sepsis: results of an interim analysis of a randomized multicentre clinical trial. Intensive Care Med 29: 834-840.
22. Zheng YM, Li F, Zhang MM, Wu XT (2006) Glutamine dipeptide for parenteral nutrition in abdominal surgery: a meta-analysis of randomized controlled trials. World J Gastroenterol 12: 7537-7541.
23. Heyland DK, Novak F, Drover JW, Jain M, Su X, et al. (2001) Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. Jama 286: 944-953.
24. Cynober L (1994) Can arginine and ornithine support gut functions? Gut 35: S42-45.
25. Stechmiller JK, Treloar D, Allen N (1997) Gut dysfunction in critically ill patients: a review of the literature. Am J Crit Care 6: 204-209.
26. Wijnands KA, Brink PR, Weijers PH, Dejong CH, Poeze M (2012) Impaired fracture healing associated with amino acid disturbances. Am J Clin Nutr 95: 1270-1277.
27. Debats IB, Wolfs TG, Gotoh T, Cleutjens JP, Peutz-Kootstra CJ, et al. (2009) Role of arginine in superficial wound healing in man. Nitric Oxide 21: 175-183.

28. Barr FE, Tirona RG, Taylor MB, Rice G, Arnold J, et al. (2007) Pharmacokinetics and safety of intravenously administered citrulline in children undergoing congenital heart surgery: potential therapy for postoperative pulmonary hypertension. J Thorac Cardiovasc Surg 134: 319-326.
29. Smith HA, Canter JA, Christian KG, Drinkwater DC, Scholl FG, et al. (2006) Nitric oxide precursors and congenital heart surgery: a randomized controlled trial of oral citrulline. J Thorac Cardiovasc Surg 132: 58-65.
30. Romero MJ, Platt DH, Caldwell RB, Caldwell RW (2006) Therapeutic use of citrulline in cardiovascular disease. Cardiovasc Drug Rev 24: 275-290.